Neonatal sensitivity to vocal emotions: A milestone at 37 weeks of gestational age

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Running title: Early emotional milestone in neonates at 37 weeks GA

Conflict of interest disclosure. The authors declare that there are no conflicts of interest in relation to this study.

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Data availability statement. The experimental materials are accessible as supplementary files for download. For further details on how to request access to EEG data and code, please contact the corresponding author, Professor D. Zhang, at zhangdd05@gmail.com.

43   Ethics approval statement & clinical trial registration. The research obtained approval from the
44   Chinese Clinical Trial Registry (ChiCTR2300069898).
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47   Subject consent statement. Prior to data collection, written consent was obtained from the parents
48   or legal guardians of all participating neonates for access to clinical information and EEG data
49   collection for scientific purposes.
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Funding statement. This study was funded by the National High Level Hospital Clinical Research
Funding (High Quality Clinical Research Project of Peking University First Hospital, 2022CR68),
the National Natural Science Foundation of China (32271102; 31920103009), the Major Project of
National Social Science Foundation (20&ZD153), Shenzhen-Hong Kong Institute of Brain
Science (2024SHIBS0004), and the National Key Research and Development Program of China
(2021YFC2700700).

Abstract

Emotional responsiveness in neonates, particularly their ability to discern vocal emotions, plays an
evolutionarily adaptive role in human communication and adaptive behaviors. The developmental
trajectory of emotional sensitivity in neonates is a crucial area of inquiry for understanding the
foundations of early social-emotional functioning. However, the precise onset of this sensitivity in
neonates and its relationship with gestational age (GA) remain subjects of investigation. In a study
involving 120 healthy neonates categorized into six groups based on their GA (ranging from 35
and 40 weeks), we delved into their emotional responses to vocal stimuli. These stimuli
encompassed disyllables with happy and neutral prosodies, alongside acoustically matched
nonvocal control sounds. The assessments occurred during natural sleep states in neonates,
utilizing the odd-ball paradigm and event-related potentials. The results unveil a distinct
developmental milestone at 37 weeks GA, marking the point at which neonates exhibit heightened
perceptual acuity for emotional vocal expressions. This newfound ability is substantiated by the
presence of the mismatch response, akin to an initial form of adult mismatch negativity, elicited in
response to positive emotional vocal prosody. Notably, this perceptual shift's specificity becomes
evident when no such discrimination is observed in acoustically matched control sounds. Neonates
born before 37 weeks GA do not display this level of discrimination ability. This critical
developmental milestone carries significant implications for our understanding of early
social-emotional development, shedding light on the role of gestational age in shaping early
perceptual abilities. Moreover, it introduces the potential for a valuable screening tool in the
context of autism, which is characterized by atypical social-emotional functions. This study makes
a substantial contribution to the broader field of developmental neuroscience and holds promise
for early intervention in neurodevelopmental disorders.

Keywords: vocal emotion, neonate, preterm, gestational age, mismatch negativity

Significance statement

This study illuminates a key developmental milestone, pinpointing the emergence of heightened
emotional perceptual acuity at 37 weeks of gestational age. Employing rigorous methods, we
reveal that neonates at this stage exhibit remarkable discrimination abilities for emotional vocal
prosody, a vital turning point in early social-emotional functioning. These findings emphasize the
pivotal role of gestational age in shaping neonatal perception and provides a pathway for early
screening of neurodevelopmental disorders, particularly autism. This insight holds profound
implications for understanding the foundations of early social-emotional development in humans,
offering a potential tool for early intervention in neurodevelopmental disorders, thereby enhancing
child health and well-being.

Introduction

Emotions represent a fundamental aspect of human social interaction, serving as a compelling subject of inquiry within the disciplines of neuroscience, psychology, and psychiatry. Over the course of evolution, the human brain has evolved to possess a heightened sensitivity to the emotional expressions of others (Lindquist et al., 2012). Remarkably, even prior to the full maturation of their visual system, human infants exhibit a remarkable ability to discern vocal emotions (Blasi et al., 2011; Soderstrom et al., 2017; Vaish & Striano, 2004). Prosodic elements of speech, including pitch, intensity, and rhythm, function as universal and non-linguistic channels for emotional communication (Latinus & Belin, 2011). Numerous studies have established that infants, including those who have not yet acquired language, exhibit differentiated responses to emotional prosody conveying happiness, fear, anger, and sadness within the age range of 2 to 12 months (e.g., Caron et al., 1988; Fernald, 1993; Graham et al., 2013; Grossmann et al., 2010; Singh et al., 2002; Walker-Andrews & Grolnick, 1983; Zhao et al., 2021).

More specifically, during the very early stages of postnatal life, often termed the neonatal period (encompassing infants under four weeks of age), compelling evidence points to the presence of emotion-specific responses to emotional cues conveyed through vocal prosody. These responses have been identified through various measurement methods, including assessments of eye-opening scores (Mastropieri & Turkewitz, 1999), event-related potentials (Cheng et al., 2012), and near-infrared spectroscopy (Zhang et al., 2019). However, prior research has primarily focused on the perception and discrimination of emotions among traditionally defined term neonates, a group that includes infants born within a five-week span (37 to 41 weeks) of gestational age (GA), treating them as a homogenous cohort. This raises a crucial question: when does emotional sensitivity begin to manifest in newborns? Does it exist in preterm neonates (GA < 37 weeks)? And does it vary among neonates born at early term (GA = 37-38 weeks) and full term (GA = 39-40 weeks), as defined by the refined 'term' classification (Spong, 2013)? Surprisingly, to date, no study has explored emotion processing in neonates with varying GAs. The discovery of this developmental milestone not only advances our understanding of the cognitive mechanisms underlying human social-emotional functioning but also provides valuable insights for early diagnosis of neurodevelopmental disorders, such as autism (Jones et al., 2014; Molnar-Szakacs et al., 2021).

The principal objective of this study is to investigate emotional responses in neonates across a range of GAs, spanning from 35 to 40 weeks, and to determine whether their heightened sensitivity to emotional voices is influenced by GA. To achieve this, we utilized the odd-ball paradigm in conjunction with an event-related potential (ERP) component known as mismatch negativity (MMN) to probe the neurobiological encoding of emotional voices in the neonatal brain. MMN is an auditory ERP component that demonstrates a negative shift in response to deviant sounds when compared to standard sounds (Näätänen et al., 2007). Importantly, it can be elicited without requiring the subject's attention, making it particularly suitable for recording in young infants (Cheour et al., 1998, 2002). It is worth noting that in neonates, this ERP component often

168 manifests as a positive response rather than the traditional MMN (e.g., Cheng et al., 2012;
169 Chládková et al., 2021; Kostilainen et al., 2020; Richard et al., 2022; Thiede et al., 2019; Virtala et
170 al., 2022; Winkler et al., 2003, 2009), leading many researchers to refer to it as the mismatch
171 response (MMR) in the neonatal brain.
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In our study, we exposed neonates to speech samples characterized by positive (i.e., happy) and
neutral prosodies. Our selection of positive emotions over negative ones (e.g., fear, sadness, or
anger) was guided not only by ethical considerations but also by previous research indicating an
early preference for positive emotions in neonates (Farroni et al., 2007; Mastropieri & Turkewitz,
1999; Zhang et al., 2019; with the exception of Cheng et al., 2012). Additionally, to eliminate the
possibility of neonates distinguishing emotional voices solely based on their low-level acoustic
features, we included another set of control sounds. These nonvocal stimuli were meticulously
matched with their vocal prosodic counterparts in terms of mean intensity and fundamental
frequency (Cheng et al., 2012). Consequently, our primary objective is to pinpoint the
developmental stage (i.e., the GA group) at which the discrimination between happy and neutral
stimuli becomes apparent for emotional voices while remaining absent for acoustically matched
control sounds.
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Results

The MMR was extracted using ERP difference waves, computed by subtracting the ERP evoked
by the standard stimulus (neutral sound) from the ERP evoked by the deviant stimulus (happy
sound) (Näätänen et al., 2007). Brain electrical activity was recorded from the F3, F4, C3, C4, P3,
and P4 sites following the international 10/20 system. However, this study primarily focused on
data from electrodes F3 and F4, as the neonatal MMR exhibits a frontal distribution (Cheng et al.,
2012; Cheour et al., 2002). Figure 1 displays MMR waveforms recorded from all six electrodes.
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Initially, we conducted a three-way repeated measures ANOVA on the mean MMR amplitudes
(time window: 150 ms to 400 ms after sound onset) with factors including condition
(vocal/nonvocal), hemisphere (left/right frontal, i.e., F3/F4) as within-subjects factors, and
neonatal group (GA = 35, 36, 37, 38, 39, and 40 weeks) as the between-subjects factor. However,
neither the main effect nor the interaction effects involving the hemisphere factor were statistically
significant (for detailed statistics, please refer to the supplemental material titled "Result of the
three-way ANOVA"). Consequently, we removed the hemisphere factor and averaged the MMR
waveforms recorded at the F3 and F4 electrodes.
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Subsequently, we performed a two-way repeated measures ANOVA with condition and group as
the two factors. The main effect of stimuli was significant, $F(1,114) = 38.827$, $p < 0.001$, $\eta_p^2 =$
0.254. Specifically, vocal stimuli elicited larger MMRs (mean ± standard deviation: 3.839 ± 4.855
µV) compared to nonvocal stimuli (0.496 ± 4.779 µV). The main effect of group was also
significant, $F(5,114) = 3.228$, $p = 0.009$, $\eta_p^2 = 0.124$. In general, MMR amplitudes were smaller
in the GA35 (0.590 ± 4.579 µV) and GA36 (0.141 ± 4.807 µV) groups compared to the GA37
(2.801 ± 5.585 µV), GA38 (2.760 ± 4.382 µV), GA39 (3.401 ± 4.871 µV), and GA40 groups

(3.311 ± 5.491 μV). However, no significant differences were found in pairwise comparisons after

Bonferroni adjustment for multiple comparisons.

The interaction between stimuli and group was significant, $F(5,114) = 3.127$, $p = 0.011$, $\eta_p^2 =$

0.121 (as shown in Figure 2). Simple effect analysis revealed that MMR amplitudes were larger in

the vocal condition compared to the nonvocal condition in the GA37 ($F(1,114) = 15.254$, $p <$

0.001, $\eta_p^2 = 0.118$; vocal = 5.367 ± 5.165 μV, nonvocal = 0.235 ± 4.847 μV), GA38 ($F(1,114) =$

16.072, $p < 0.001$, $\eta_p^2 = 0.124$; vocal = 5.394 ± 3.145 μV, nonvocal = 0.126 ± 3.861 μV), GA39

($F(1,114) = 8.393$, $p = 0.005$, $\eta_p^2 = 0.069$; vocal = 5.305 ± 4.011 μV, nonvocal = 1.498 ± 4.998

μV), and GA40 groups ($F(1,114) = 14.482$, $p < 0.001$, $\eta_p^2 = 0.113$; vocal = 5.811 ± 5.298 μV,

nonvocal = 0.811 ± 4.546 μV). However, there were no significant differences in MMR

amplitudes between the two kinds of stimuli in the GA35 ($F(1,114) = 0.026$, $p = 0.873$, $\eta_p^2 <$

0.001; vocal = 0.695 ± 4.031 μV, nonvocal = 0.485 ± 5.173 μV) and GA36 groups ($F(1,114) =$

0.236, $p = 0.628$, $\eta_p^2 = 0.002$; vocal = 0.460 ± 4.104 μV, nonvocal = -0.179 ± 5.511 μV).

Further analysis revealed that vocal stimuli evoked varying MMR amplitudes across groups,

$F(5,114) = 6.768$, $p < 0.001$, $\eta_p^2 = 0.229$. Specifically, the MMRs evoked by vocal stimuli were

smaller in the GA35 group compared to GA37 ($p = 0.014$), GA38 ($p = 0.013$), GA39 ($p = 0.017$),

and GA40 groups ($p = 0.005$). Similarly, the MMRs evoked by vocal stimuli were smaller in the

GA36 group compared to GA37 ($p = 0.008$), GA38 ($p = 0.008$), GA39 ($p = 0.009$), and GA40

groups ($p = 0.003$). However, nonvocal stimuli did not elicit significantly different MMR

amplitudes across groups, $F(5,114) = 0.300$, $p = 0.912$, $\eta_p^2 = 0.013$.

Figure 1. Mismatch response (MMR) waveforms recorded across six electrodes in different
gestational age (GA) groups. The MMR is extracted by subtracting the event-related potential
(ERP) elicited by the standard stimulus (neutral condition) from the ERP evoked by the deviant
stimulus (happy condition).
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Figure 2. Primary MMR results. A, ERP waveforms (averaged at the F3 and F4 electrodes) across the six GA groups. The time window for assessing MMR amplitude is indicated between the two vertical gray lines. B, Violin plot illustrating MMR amplitudes in the six GA groups. Simple effect analysis was performed with pairwise comparisons, corrected using the Bonferroni method: ** $p <$ 0.010, *** $p < 0.001$.

Discussion

The current study elucidates a pivotal developmental milestone in neonatal emotional responsiveness by investigating their ability to perceive vocal emotions. The findings illuminate a distinct turning point at 37 weeks of gestational age (GA), representing the onset of heightened perceptual acuity for emotional vocal expressions. This milestone is particularly evident in the robust MMR to positive emotional vocal prosody. Significantly, the absence of this discrimination ability when acoustically matched control sounds were presented underscores the specificity of this developmental shift towards emotional voice processing. Our identification of the 37-week GA mark aligns with previous research, which indicated emotional sensitivity in term neonates born at or after 37 weeks of gestation (Cheng et al., 2012; Farroni et al., 2007; Mastropieri & Turkewitz, 1999; Zhang et al., 2019) and in preterm neonates (GA < 37 weeks) tested at term age (Kostilainen et al., 2020). Notably, our findings also reveal that neonates born before 37 weeks GA do not exhibit these emotional discrimination abilities.

In the final trimester of pregnancy, the human brain undergoes a period of rapid and continuous changes in neural structure and cognitive functions (Bayer et al., 1993; Clancy et al., 2007). Although there is no direct evidence to support the notion that preterm neonates can decode vocal emotions, they have displayed an aptitude for processing speech stimuli with social contexts. For

example, neonates born at or after 29 weeks GA have shown a preference for infant-directed speech, characterized by a high pitch, exaggerated pitch modifications, and a slow rate (Butler et al., 2014). This preference has been associated with increased visual attention, heightened alertness (Eckerman et al., 1994), reduced heart rate (White-Traut et al., 1997), and enhanced speech differentiation in premature babies (Richard et al., 2022). Additionally, neonates born at or after 30 weeks GA have demonstrated an age-related increase in sensitivity to maternal voices (D Chorna et al., 2018; A. P. F. Key et al., 2012), leading to beneficial effects on cognitive and neurobehavioral development (Caskey et al., 2011; Picciolini et al., 2014; see Provenzi et al., 2018 for a review). These effects encompass improved feeding behaviors, heightened responsiveness (Katz, 1971; Krueger et al., 2010), enhanced weight gain (Zimmerman et al., 2013), and activated auditory cortical plasticity (Webb et al., 2015). Both infant-directed speech and maternal voices feature extensive pitch modulation, and the preference for these emotionally prosodic-like voices during the preterm stage may prepare the developing brain to discriminate vocal emotions at 37 weeks GA, as demonstrated in this study.

Traditionally, 37 weeks of gestation served as the benchmark for fetal maturity, and term infants born within the 37 to 41 weeks GA range were generally considered healthy, forming a homogenous group. Recent insights, however, have unveiled variations in physical and cognitive maturation within this 5-week span of full-term pregnancy. Research indicates that neonates born at 37-38 weeks GA face increased risks of neonatal mortality and pediatric respiratory, neurologic, and endocrine morbidities compared to those born at 39-41 weeks GA (Cahen-Peretz et al., 2022; Clark et al., 2009; Edwards et al., 2013; Ghartey et al., 2012; Paz Levy et al., 2017; Sengupta et al., 2013; Tita et al., 2009). Furthermore, a dose-response relationship inversely linking GA to the risk of developmental delay has been identified in infants from preterm to full-term births (Rose et al., 2013; Schonhaut et al., 2015). Early birth (34-38 weeks GA) has also been found to have a detrimental impact on child development and academic achievement during school age (Bentley et al., 2016; Chan et al., 2016; Dong et al., 2012; Hedges et al., 2021; Murray et al., 2017; Nielsen et al., 2019; Noble et al., 2012). Consequently, the definition of a full-term pregnancy has been narrowed to a two-week window starting at 39 weeks (Spong, 2013), with nonmedically indicated deliveries between 37 and 38 weeks of gestation discouraged (ACOG Committee Opinion, 2019). While accumulating evidence underscores the adverse effects of the traditional 37-week threshold, our findings contribute to a limited body of research suggesting that neonatal social-emotional functioning reaches a development milestone at 37 weeks GA.

A more comprehensive understanding of the developmental trajectory of emotional sensitivity has the potential to revolutionize decision-making in the final weeks of pregnancy and the identification of newborns at risk of emotional and neurodevelopmental disorders, particularly autism. Individuals with autism often exhibit atypical perceptual and neural processing of emotional information, including emotional prosodic voices (Kuhl et al., 2005; Lindström et al., 2018; Van Lancker et al., 1989; Wang et al., 2007; for comprehensive reviews, see Frühholz & Staib, 2017; Yeung, 2022). While previous studies have indicated that social-emotional behavioral indicators typically begin to demonstrate predictive power for autism from the second year of life (Gliga et al., 2014; Jones et al., 2014), brain functional indicators of emotional processing during infancy, especially within the first year of life, have already shown their predictive value (Ayoub

et al., 2022; Clairmont et al., 2021; Molnar-Szakacs et al., 2021). For instance, infants subsequently diagnosed with autism displayed a smaller amplitude and shorter duration of the negative central (Nc) component at six months of age when viewing smiling faces compared to toys, a pattern not observed in infants who were subsequently undiagnosed (Jones et al., 2016). Additionally, while the Nc and P400 components were able to distinguish between smiling, fearful, and neutral facial expressions in typically developing 9-to-10-month-old infants, these EEG indicators failed to differentiate emotional faces in infants at high risk for autism (Di Lorenzo et al., 2021; A. P. Key et al., 2015). Furthermore, it was observed that infants at high risk for autism exhibited reduced activation in the fusiform gyrus and hippocampus compared to healthy controls in response to sad cries between 4 and 7 months of age (Blasi et al., 2015). Building upon these existing studies, our research introduces a promising early screening indicator for autism: the neonatal MMR in response to emotional voices. We recommend future longitudinal studies to further elucidate the predictive role of this neurophysiological indicator, thereby facilitating early diagnosis and intervention for social-emotional disorders.

Furthermore, there is a compelling need for future investigations to expand upon the present findings by incorporating a broader array of emotional stimuli. Non-speech emotional vocalizations, including laughter, crying, or retching, as well as natural emotional auditory cues such as thunder, flowing water, hissing snakes, and bird calls, present a rich spectrum of emotional materials that have been shown to captivate the perceptual faculties of neonates and infants (Blasi et al., 2011; Erlich et al., 2013). This multifaceted approach could illuminate whether the developmental milestone observed at 37 weeks GA is specific to the processing of emotional prosodic speech and vocal expressions, or if it extends to encompass a broader range of both artificial and natural emotional auditory cues. Additionally, the inclusion of non-speech emotional materials may offer insights into the potential right lateralization of emotional processing in the neonatal brain. While prior studies (including some cited herein) have identified right lateralization for emotional processing in full-term neonates (Cheng et al., 2012; Zhang et al., 2019;see Bisiacchi & Cainelli, 2022 for a comprehensive review), the introduction of non-speech materials can help disentangle the confounding effects of left lateralization, which is associated with language processing and has been identified in both preterm (Mahmoudzadeh et al., 2013) and full-term neonates (Kotilahti et al., 2010; May et al., 2018; Peña et al., 2003; Sato et al., 2012; Vannasing et al., 2016; Wu et al., 2022).

In summary, this study has illuminated a pivotal developmental milestone – the emergence of heightened perceptual acuity for emotional vocal expressions at 37 weeks GA. It is important to note that neonates' perceptual sensitivity at this stage is unlikely to be associated with a profound conceptual understanding of the meaning of emotions. Nevertheless, this unique discrimination ability in early life may serve as a foundational building block for the subsequent development of emotional and social cognition. Beyond its scientific significance, our findings underscore the critical role of gestational age in shaping early perceptual abilities and offer a promising avenue for early screening and intervention in neurodevelopmental disorders, particularly autism, where early detection is of paramount importance. This work not only deepens our understanding of neonatal social and emotional development but also provides a potential tool to support early diagnosis and intervention in this critical realm of child health and well-being.

Materials and methods

Subjects

The research obtained approval from both the Ethical Committee of Peking University First Hospital and the Chinese Clinical Trial Registry (ChiCTR2300069898). We invited 120 healthy neonates, of which 60 were boys, to participate within the first 24 hours after birth (mean ± standard deviation: 17.8 ± 0.4 hours). These neonates were categorized into six groups based on their GA, specifically 35, 36, 37, 38, 39, and 40 weeks, each consisting of twenty subjects. For example, the GA35 group comprised neonates whose GA ranged from 35 weeks plus 0 day to 6 days. While sample sizes were not statistically predetermined, recruiting twenty subjects per GA group represented the maximum feasible number within a two-year period at Peking University First Hospital.

All subjects met the following inclusion criteria: 1) normal birth weight for their GA; 2) absence of clinical symptoms at the time of EEG recording; 3) no previous sedation or medication prior to EEG recording; and 4) normal hearing results in an evoked otoacoustic emissions test (ILO88 Dpi, Otodynamics Ltd, Hatfield, UK). Additionally, subjects did not exhibit any of the following neurological or metabolic disorders: 1) hypoxic-ischemic encephalopathy, 2) intraventricular hemorrhage or white matter damage detected by cranial ultrasound, 3) congenital malformation, 4) central nervous system infection, 5) metabolic disorder, 6) clinical evidence of seizures, and 7) signs of asphyxia. Prior to data collection, written consent was obtained from the parents or legal guardians of all participating neonates for access to clinical information and EEG data collection for scientific purposes.

Stimuli

A total of 85 possible combinations of consonants and vowels, which are standard in Chinese (Lee & Zee, 2003) and common to most human languages (e.g., 'dada' and 'keke'), were recorded by a native Chinese-speaking adult woman with the Peking dialect. Each disyllable was recorded with four repetitions, two using a happy prosody and two with a neutral prosody, resulting in a total of 340 disyllables (85 × 4). Twenty Chinese undergraduate students (10 men, mean age 20.1 ± 1.2 years) performed a discrimination task, distinguishing between happy and neutral stimuli, and rated the affective content of these stimuli.

In the affective rating task, participants assessed the intensity of happiness (on a 9-point scale ranging from 1 being the least happy to 9 being the happiest) and the valence (on a 9-point scale ranging from 1 being the most negative, 5 being neutral, to 9 being the most positive) of the 340 stimuli. This study selected five pairs of happy and neutral disyllables that shared the same consonant-monophthong combinations and achieved 100% discrimination accuracy in the discrimination task (i.e., 'dada', 'dudu', 'gege', 'keke', and 'tutu' in Chinese Pinyin). Paired-samples t-tests demonstrated that the happy disyllables were rated as significantly happier ($t(4) = 24.70$, $p < 0.001$; happy intensity: 7.49 ± 0.20 versus 3.53 ± 0.19) and had a more positive

valence ($t(4)$ = 18.55, p < 0.001; valence: 7.11 ± 0.12 versus 4.91 ± 0.24) than their neutral counterparts. These ten disyllables were then standardized to have the same mean intensity and had duration of 400 ms using Adobe Audition (v.2022; Adobe Systems Inc., San Jose, CA).

To ensure that neonates were discriminating based on prosodic cues containing emotional content rather than low-level acoustic properties, we employed a method similar to Cheng et al. (2012) and created a separate set of nonvocal control sounds. We hypothesized that the fundamental frequency (f0) alone does not convey emotional content in voices and that neonates require multiple other prosodic cues embedded in the high-frequent component to discern emotions, as suggested by Cheng et al. (2012) and Zhang et al. (2014). As a result, ten nonvocal sounds were generated to match the f0 contours and temporal envelopes of their corresponding vocal sounds. This matching process was performed using Praat (v.6.3.17, www.praat.org) and Matlab (v.2021b; MathWorks, Inc., South Natick, MA). Oscillograms and spectrograms of the auditory stimuli used in this study are presented in Figure 3. All auditory stimuli, along with their pronunciations and rating scores, are available in the supplemental material labeled "experimental sounds".

To optimize the diversity of our material and increase the generalizability of our results, we utilized ten sets of sounds. Each set included both positive and neutral prosodic voices, along with their respective nonvocal counterparts. These auditory materials were distributed randomly and evenly within each neonatal GA group, ensuring that each set was presented twice (to two individuals) in each GA group.

Procedure

The sound stimuli were presented in two blocks: the vocal and nonvocal conditions, utilizing the odd-ball paradigm. The standard stimulus was either a vocal or nonvocal neutral sound, while the deviant stimulus was either a vocal or nonvocal happy sound. Each block consisted of 240 standard stimuli (80%) and 60 deviant stimuli (20%). The standard and deviant stimuli were presented randomly, ensuring that each deviant stimulus was followed by at least two standard stimuli. Each sound had a duration of 400 ms, and the inter-trial interval was silent, with varying durations ranging from 500 to 700 ms. Each block lasted for 5 minutes, and the order of the vocal and nonvocal blocks was counterbalanced across participants. A 5-minute break separated the two blocks, resulting in a total EEG recording duration of 15 minutes.

The experiment took place in the neonatal ward of Peking University First Hospital. Neonates were transported to a dedicated testing room for EEG recording. Auditory stimuli were presented through a pair of loudspeakers positioned approximately 30 cm away from the neonates' left and right ears, at a sound pressure level of 55 to 60 dB, with an average background noise intensity level of 30 dB. EEG recording was conducted while the neonates were in a natural sleep state (Cheour et al., 2002; Wu et al., 2022).

Data recording and analysis

We recorded brain electrical activity using an electrical amplifier (NeuSen.W32, Neuracle, Changzhou, China) at a sampling frequency of 1000 Hz. Initially, the data were recorded online with reference to the left mastoid and subsequently re-referenced offline to the average of the left and right mastoids. The ground electrode was positioned on the forehead. For the recording of vertical eye movements, an electrooculogram (EOG) electrode was positioned beneath the left eye, while another was placed at the left external canthi for recording horizontal eye movements. Throughout the recording process, electrode impedances were meticulously maintained below 10 kΩ.

We eliminated ocular artifacts from the EEG data using a regression procedure implemented in NeuroScan software (Scan 4.3, NeuroScan, Herndon, VA). Subsequently, we employed Matlab (v.2021b; Mathworks, Inc., Sherborn, MA) for data processing and result presentation. The EOG-corrected EEG data were then offline filtered with a half-amplitude cutoff range of 0.01~30 Hz and segmented from 200 ms before sound presentation until 1000 ms after sound onset. Epochs were baseline-corrected relative to the mean voltage during the 200 ms preceding sound presentation. Epochs containing artifacts with peak deflections exceeding ±200 µV were rejected, followed by averaging for each experimental condition. The number of valid epochs did not exhibit a significant difference across neonatal groups (please refer to supplemental material "Epoch number"). The time window for the MMR component was pre-defined as 150 ms to 400 ms after sound onset, based on prior knowledge (Cheour et al., 2002), and utilized throughout the data analysis.

We performed statistical analyses using SPSS Statistics (v. 20.0; IBM, Somers, USA). Descriptive data are reported as mean ± standard deviation. The significance level was set at 0.05. We applied the Greenhouse-Geisser correction for ANOVA tests when deemed appropriate. Post-hoc tests for significant main effects were conducted using the Bonferroni method. Significant interactions were explored through simple effects models. We reported partial eta-squared (η_p^2) as a measure of effect size in ANOVA tests.

Figure 3. Oscillograms and spectrograms displaying vocal and nonvocal auditory stimuli for the syllable 'keke' (international phonetic symbol: [k□□ k□□]).

Acknowledgments

This study was funded by the National High Level Hospital Clinical Research Funding (High Quality Clinical Research Project of Peking University First Hospital, 2022CR68), the National Natural Science Foundation of China (32271102; 31920103009), the Major Project of National Social Science Foundation (20&ZD153), Shenzhen-Hong Kong Institute of Brain Science (2024SHIBS0004), and the National Key Research and Development Program of China (2021YFC2700700).

Data and code availability

The experimental materials are accessible as supplementary files for download. Additional data and the code used in this study will be made available upon reasonable request, subject to approval from the Institute of Brain and Psychological Sciences at Sichuan Normal University. For further details on how to request access to EEG data and code, please contact the corresponding author, Professor D. Zhang, at zhangdd05@gmail.com.

Author contributions

D. Zhang and X. Hou conceptualized and designed the research. P. Zhang, X. Hou, and C. Peng conducted the experiments, while P. Zhang, L. Mo, and D. Zhang were responsible for data analysis. D. Zhang authored the paper, and X. Hou revised and provided critical input to the manuscript.

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vocal happy 'keke'

vocal neutral 'keke'

nonvocal happy 'keke'

nonvocal neutral 'keke'

dB/Hz

-50

-100

-150

frequency (kHz)

100   200   300

time (ms)